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PAPER

Safety, pharmacokinetics, and pharmacodynamics of RSLV-132, an RNase-Fc fusion protein in systemic lupus erythematosus: a randomized, double-blind, placebo-controlled study

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Blood-borne RNA circulating in association with autoantibodies is a potent stimulator of interferon production and immune system activation. RSLV-132 is a novel fully human biologic Fc fusion protein that is comprised of human RNase fused to the Fc domain of human IgG1. The drug is designed to remain in circulation and digest extracellular RNA with the aim of preventing activation of the immune system via Toll-like receptors and the interferon pathway. The present study describes the first clinical study of nuclease therapy in 32 subjects with systemic lupus erythematosus. The drug was well tolerated with a very favorable safety profile. The approximately 19-day serum half-life potentially supports once monthly dosing. There were no subjects in the study that developed anti-RSLV-132 antibodies. Decreases in B-cell activating factor correlated with decreases in disease activity in a subset of patients. *Lupus* (2016) **0,** 1–10.

Key words: RNA immune complex; nuclease therapy; interferon

Introduction

Impaired clearance of apoptotic cell debris is thought to trigger the pro-inflammatory cascade characteristic of systemic lupus erythematosus (SLE). The loss of self-tolerance and the development of autoantibodies to certain autoantigens is a key serologic feature of SLE.[1,2] Many autoantibodies such as anti-SSA/Ro, anti-SSB/La, anti-SM, and anti-RNP recognize nuclear antigens which are involved in RNA processing or transcription and are complexed with RNA.[3,4] The recognition that autoantibodies, by virtue of their Fc domain, deliver RNA to immune cell intracellular compartments where it is exposed to and activates Toll-like receptors (TLRs) advanced the understanding of how autoantibodies perpetuate autoimmunity in SLE.[5-9] Given that autoantibody delivery of RNA to intracellular TLRs is the initial event which triggers a broad cascade of pro-inflammatory biology, removal of RNA from autoantibodies would be expected to have a global dampening effect on the overall state of inflammation in SLE. Therefore, RSLV-132 was developed to enzymatically digest autoantibody-bound RNA, thereby rendering the autoantibodies unable to stimulate intracellular inflammatory pathways. RSLV-132 is a fully human Fc fusion protein comprised of human RNase fused to the amino terminus of the Fc domain of human IgG1. The present study is the first report of the effect of RSLV-132 in patients with SLE and summarizes the safety, pharmacokinetic, and pharmacodynamic data from a multiple escalating dose study of RLSV-132 in SLE subjects.

Patients and methods

Study design

This was a multicenter phase 1b, randomized, double-blind, placebo-controlled, dose escalation study of three to five intravenous infusions of

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RSLV-132 over one month in adults with SLE (ClinicalTrials.gov identifier: NCT02194400). The primary goal of the study was to evaluate the safety and tolerability of multiple doses of RSLV-132 in subjects with mild, stable SLE. The study tested five weekly doses, or three bi-weekly doses of 0.3 mg/kg to 10.0 mg/kg of RSLV-132 and consisted of a 28-day screening period followed by 30 days of treatment and a 30–90 day follow up period. Incremental dose escalation occurred following the review of blinded safety data by an independent data safety monitoring board in all of the subjects in a given cohort after two weeks of exposure. A total of 32 subjects were enrolled into the study. Eight subjects were randomized at a ratio of 3:1, active treatment to placebo to each of four cohorts. Subjects in cohort 1 were administered placebo weekly for a total of five doses or 0.6 mg/kg on Day 1, followed by four weekly doses of 0.3 mg/kg RSLV-132. Subjects in cohort 2 were administered placebo every other week for three doses or 2.0 mg/kg of RSLV-132 followed by two doses of 1.0 mg/kg administered every other week. Subjects in cohort 3 were administered placebo every other week for three doses or 6.0 mg/kg of RSLV-132 followed by two doses of 3.0 mg/kg RSLV-132 administered every other week. Subjects in cohort 4 were administered three doses of 10.0 mg/kg RSLV-132 or placebo administered every other week. In cohort 1, intravenous infusions occurred on Days 1, 8, 15, 22, and 29. In cohorts 2–4, infusions took place on Days 1, 15, and 29. Subjects were allowed to continue the use of their SLE medications without changes in dose or addition or removal of medications.

Patients

The study was conducted at five rheumatology centers in the United States. Subjects enrolled in the study were between the ages of 18 and 70, met four of the 11 American College of Rheumatology revised classification criteria for SLE,[10,11] and had confirmed antibody-bound U1 or Y1 RNA significantly higher than the levels observed in healthy volunteers using a semi-quantitative protein-A capture, rt-qPCR assay. Patients enrolled in the study had primarily inactive to mild disease and were not expected to have changes in their SLE medications in the coming 30 days. The primary exclusion criteria included SLE manifestations affecting major organ systems; use of cyclophosphamide, rituximab, belimumab, or any other biological agent within 180 days of screening; use of prednisone or corticosteroid equivalent at a dose greater than or equivalent to 10 mg/day; or the presence of acute infection within seven days prior to baseline; or participation in another clinical trial within 12 weeks or five half-lives of the study drug, whichever was longer. SLE medications were to be kept constant during the one-month study period. Written informed consent was obtained from all study subjects. The study was conducted in accordance with the International Conference on Harmonization Guidance for Good Clinical Practice and the Declaration of Helsinki.

Pharmacokinetic analysis

For cohort 1, blood samples for pharmacokinetic (PK) analyses were obtained on Day 1 at pre-dose, 0.5, 1, 4, 8, 24, 72, and 168 hours post-infusion, pre-dose on Days 15 and 22, and on Day 29 at pre-dose, 0.5, 1, 4, 8, 24, 72, 168, 336, and 672 hours post-infusion. In cohorts 2–4, PK samples were obtained on Day 1 at pre-dose, 0.5, 1, 4, 8, 24, 168, and 336 hours post-infusion, pre-dose on Day 15, and on Day 29 at pre-dose, 0.5, 1, 4, 8, 24, 168, 336, 672, and 2016 hours post-infusion. The samples were processed to serum and RSLV-132 concentrations were measured using a validated competitive enzyme-linked immunosorbent assay (ELISA) method. Given that the mechanism of action of RSLV-132 involves its RNase catalytic activity, a qualified RNase catalytic activity assay method was developed using the RNaseAlert system (Life Technologies) modified for use in human serum. The RNase activity assay was used to measure the serum RNase catalytic activity at the same time points as the ELISA measurements. Since pre-dose baseline RNase activity was observed, the Day 1 pre-dose RNase activity value for each subject was subtracted from all of the reported values generated from the RNase assay for that subject prior to PK analyses. The Day 1 and Day 29 RSLV-132 serum concentration versus time profiles from each subject, generated using both the ELISA and RNase activity-based assays were evaluated by noncompartmental methods using WinNonlin Phoenix version 6.3 software (Certara USA, Inc., Princeton, NJ). The estimated PK parameters were the maximum observed concentration (C_{max}), time of maximum observed concentration (T_{max}), area under the curve from time 0 until the end of the dosing interval (AUC_{tau}), area under the curve from time 0 until the time point with the last measurable concentration (AUC_{0-t}), terminal half-life ($t_{1/2}$), apparent clearance (CL), and steady-state volume of

distribution (V_{ss}). Due to the long $t_{1/2}$ of RSLV-132, the terminal phase of the concentration versus time profiles could not be characterized after the Day 1 dose and parameters based on the terminal phase were not estimated. However, these parameters were generated following the final Day 29 dose. The level of accumulation observed on Day 29, relative to Day 1, was evaluated using accumulation ratios based on C_{max} and AUC_{tau}. With the exception of the 10 mg/kg dose cohort, the first dose was administered as a 2× loading dose. Therefore, exposure parameters were dose-normalized prior to the calculation of accumulation ratios. In addition to the comparison of Day 1 and Day 29 PK parameters, the pre-dose troughs obtained throughout the study were compared to those obtained after Day 1 dosing.

Autoantibody measurements

Serum was collected to measure ds-DNA, SSA/Ro, SSB/La, Sm, RNP, and U1RNP autoantibodies, C3 and C4. Serum was collected pre-dose on Days 1 and 29, and on Days 43 and 57 for cohort 1. In cohorts 2–4 serum was collected pre-dose on Days 1 and 29, and on Day 43. Anti-dsDNA autoantibodies were measured using an ELISA assay (BioRad), the other autoantibodies were measured using Bioplex 2200 kits (BioRad).

Anti-RSLV-132 antibodies

To measure anti-RSLV-132 antibodies, a validated electrochemiluminescent (ECLA) assay was developed and deployed (ICON laboratories plc) using Good Clinical Laboratory Practices (GCLP). Serum was analyzed for anti-RSLV-132 antibodies pre-dose on Day 1, and on Days 29 and 57 (cohort 1); and pre-dose on Day 1, and Days 29 and 113 (cohorts 2–4).

Gene expression analysis

Isolation, characterization of the quality of the RNA, and Fluidigm dynamic array (48×48) qPCR analysis were performed by EA Genomics Lab of Q^2 Solutions (Durham, NC). The array measured 180 transcripts which were selected based on their involvement in known inflammatory pathways and was dominated by the presence of interferon-responsive genes. In addition, genes associated with neutrophil, B-cell, and myeloid modules were also included. An interferon signature metric (ISM) was calculated for all of the subjects in the study and 20 healthy volunteers.[12]

Interferon-inducible protein analysis

Serum B-cell activating factor (BAFF) levels were measured pre-dose on Days 1 and 36, using a validated quantitative immunoassay (MyriadRBM; Austin, TX).

Disease activity

While the study was not designed or powered to demonstrate differences between the treatment groups, the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI) was used to monitor disease activity during the study. Measurements were made pre-dose on Days 1, 29, and 43.

Results

Patient demographics and disease activity

This study enrolled 32 subjects with mild, stable SLE. The baseline demographics, disease activity, serology, and background medications of the SLE patients enrolled in the study are summarized in Table 1.

Safety and tolerability

This study was designed to evaluate the safety and tolerability of three to five doses of RSLV-132 over 30 days of exposure at doses escalating from 0.3 mg/kg to 10 mg/kg. There were six (75%) subjects in the placebo group and 18 (75%) in the RSLV-132 treated group that experienced one or more adverse events (AEs). There were a total of 11 AEs (eight grade 1, three grade 2) in the placebo group, and 61 (48 grade 1, 12 grade 2, one grade 3) in the RSLV-132 treated group. The majority of AEs ($N = 44$; 73%) and the serious adverse event (SAE) ($N = 1$) were judged by the investigator as not related to the study drug. There were no infusion reactions observed during the study. The treatment-emergent AEs that occurred in two or more instances are summarized in Table 2. There was one discontinuation due to an SAE in a subject with a history of cholecystitis who experienced acute cholecystitis that developed on Day 8 of the study and required cholecystectomy. There were no deaths in the study. The frequency of AEs in the active treatment group was similar to placebo, and there was no relationship between the dose level and AEs.

Table 1 Baseline characteristics of the systemic lupus erythematosus patients

		Cohort 1 (N = 6)	*Cohort 2* (N = 6)	*Cohort 3* (N = 6)	*Cohort 4* (N = 6)	*Placebo* (N = 8)	*Total* (N = 32)
Age		40 ± 9.9	47 ± 15.1	48 ± 12.5	57 ± 7.4	43 ± 8.1	47 ± 8.1
Sex	Male	—	1 (17%)	—	2 (33%)	1 (13%)	4 (13%)
	Female	6 (100%)	5 (83%)	6 (100%)	4 (67%)	7 (88%)	28 (88%)
Race	White	4 (67%)	5 (83%)	4 (67%)	4 (67%)	6 (75%)	23 (72%)
	Black	1 (17%)	1 (17%)	2 (33%)	2 (33%)	2 (25%)	8 (25%)
	Asian	1 (17%)					
SLEDAI	Avg. (range)	5.7 (0–12)	3.0 (0–8)	2.7 (0–4)	2.3 (0–6)	3.8 (0–6)	
SLEDAI = 0		1	2	1	3	2	
ISM High	(>1)	4 (67%)	5 (83%)	6 (100%)	4 (67%)	6 (75%)	25 (78%)
	Avg. Score	2.5	1.9	3	1.7	2.1	2.2
Anti-ds-DNA		2 (33%)	1 (17%)	2 (33%)	2 (33%)	2 (33%)	9 (28%)
≥1 RNA Autoantibody		6 (100%)	3 (50%)	6 (100%)	5 (83%)	6 (75%)	26 (81%)
HCQ		3 (50%)	3 (50%)	5 (83%)	4 (67%)	7 (88%)	22 (69%)
OCS		1 (17%)	0 (0%)	1 (17%)	1 (17%)	3 (38%)	6 (19%)
MTX		1 (17%)	2 (33%)	0 (0%)	1 (17%)	1 (17%)	5 (16%)
MMF		0 (0%)	2 (33%)	1 (17%)	1 (17%)	2 (33%)	6 (19%)

ISM: interferon signature metric; HCQ: hydroxychloroquine; OCS: oral corticosteroids; MTX: methotrexate; MMF: mycophenolate mofetil.

Table 2 Treatment-emergent adverse events occurring in two or more instances (all causalities)

Adverse event	*Placebo* (N = 8)	*0.6 mg/kg × 1* *0.3 mg/kg × 4* (N = 6)	*2.0 mg/kg × 1* *1.0 mg/kg × 2* (N = 6)	*6.0 mg/kg × 1* *3.0 mg/kg × 2* (N = 6)	*10.0 mg/kg × 3* (N = 6)	*RSLV-132 Overall* (N = 24)
Headache	3					5
Grade 1	2	0	1	1	3	
Grade 2	1	0	0	0	0	
Upper respiratory infection	1					5
Grade 1	1	2	2	1	0	
Nausea						5
Grade 1	0	2	1	1	0	
Grade 2	0	1	0	0	0	
Vomiting						2
Grade 1	0	0	0	0	1	
Grade 2	0	1	0	0	0	
Gastritis	1					1
Grade 1	1	1	0	0	0	
Sinusitis						2
Grade 1			1			
Grade 2			1			
Cough						2
Grade 1	0	0	0	0	2	
Oropharyngeal pain						2
Grade 1	0	1	0	1	0	
Rash						2
Grade 1	0	1	1	0	0	
Dizziness						2
Grade 1		1			1	

Pharmacokinetics

Mean serum concentration versus time data generated from the ELISA assay and the background-corrected RNase data are shown in Figure 1. The overall shape of the PK profile and the T_{max} values were consistent between the ELISA and RNase activity assay methods. In general, RSLV-132 exposure increased in a dose-proportional to slightly higher than dose-proportional manner.

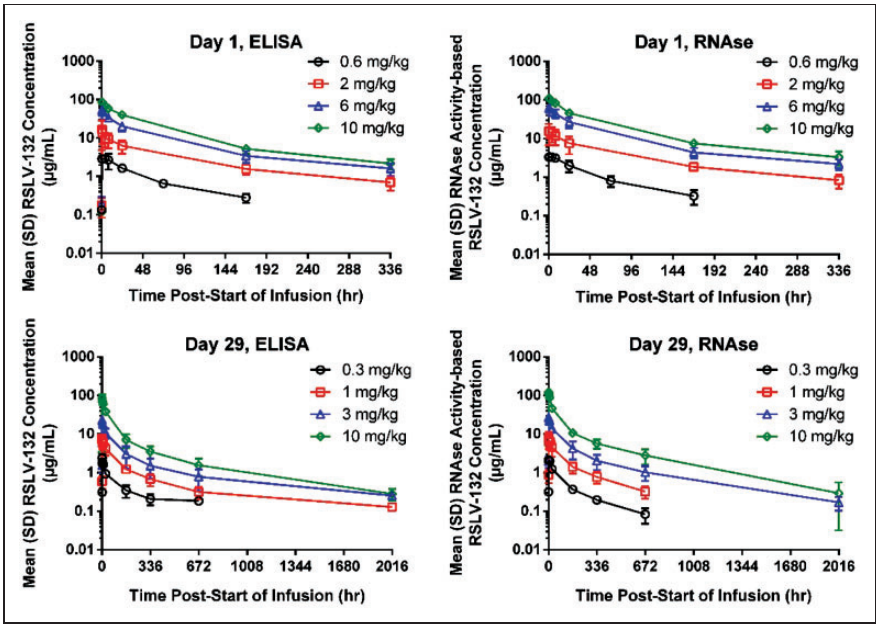

Figure 1 Mean (SD) serum RSLV-132 concentrations measured by competitive ELISA or RNase catalytic activity are shown for Day 1 and Day 29 for each cohort.

Following the Day 29 dose, the mean $t_{1/2}$ increased with dose between the 0.3 and 1 mg/kg dose levels, but plateaued between the 1, 3, and 10 mg/kg dose levels (11.2 days versus 19.0 to 25.4 days based on the ELISA data). Mean CL values for RSLV-132 ranged from 0.818 to 1.35 mL/h/kg and mean V_{ss} values ranged from 290 to 434 mL/kg. Both CL and V_{ss} values for RSLV-132 are somewhat higher than those observed for monoclonal antibodies to a circulating ligand.[13] Little to no accumulation based on dose-normalized C_{max} or AUC_{tau} values was observed in any of the cohorts. Mean accumulation ratios based on ELISA data ranged from 0.915 to 1.60, and those based on RNase activity data ranged from 0.881 to 1.73. Based on the long RSLV-132 $t_{1/2}$ and the dosing regimen, an accumulation ratio of approximately two to three would have been expected, but this level of accumulation was not observed. This less than expected accumulation is often observed for drugs that exhibit multicompartmental kinetics.[14] In addition, there was little to no accumulation observed based on predose trough concentrations suggesting steady-state was achieved over the course of the study.

Immunogenicity

Anti-RSLV-132 antibodies were measured at baseline prior to dosing, at Day 29, and at the end of study visit following the last dose of RSLV-132 (Day 57 in cohort 1, or Day 113 in cohorts 2–4). There were no subjects with anti-RSLV-132 antibodies at any time point in the study.

Gene expression profiles

The baseline profile of the 32 study subjects and 20 healthy volunteers for the 180 genes in the array are show in Figure 2. Analysis of the modules contained in the array across the four cohorts of subjects in the study demonstrated changes in gene expression that were specific for individual drug-treated subjects as opposed to placebo-treated subjects. However, there was no consistent pattern of alteration in gene expression among the drug-treated groups. This likely reflects the small sample size, the great variation among the patients, and the short duration of treatment. The baseline ISM was calculated for the 32 study subjects and 20 healthy volunteers; the overall distribution is shown in Figure 3, individual ISM scores within each cohort are shown in Figure 4. At baseline the majority of study subjects were interferon signature positive with a mean ISM of approximately three. During the study, the ISM scores remained stable. Decreases in ISM were observed for two subjects in the RSLV-132 treatment group, and none in the placebo group. Subject 112, with the highest SLEDAI in the study, had a decrease of approximately 50% at Day 29; a second subject, 308, had a decrease of 70% at Day 43, which coincided with a five-day course of steroid usage for the

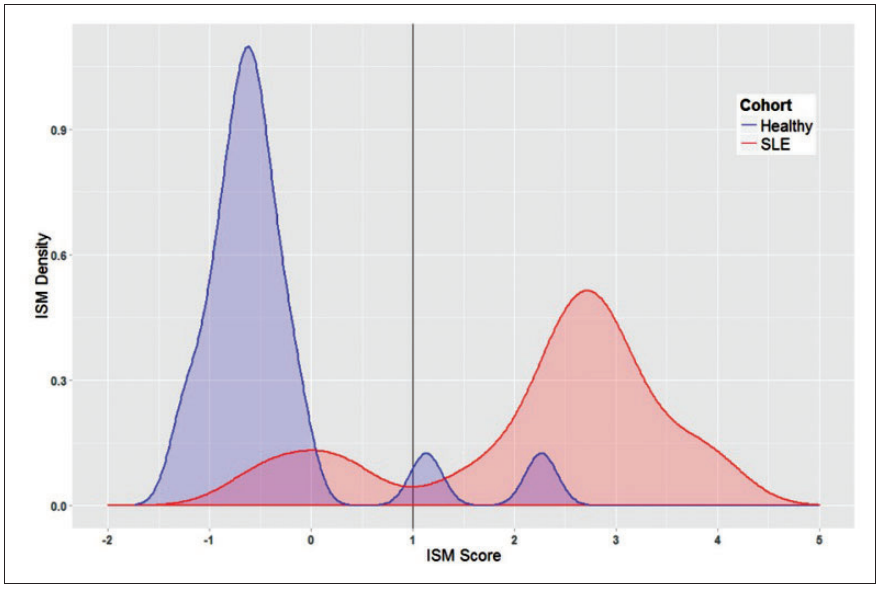

Figure 2 The ISM score for 32 study subjects was calculated at baseline. The interferon signature metric score for 20 healthy volunteers is also shown in the graph. An ISM score of ≥1 was considered to be IFN signature positive.

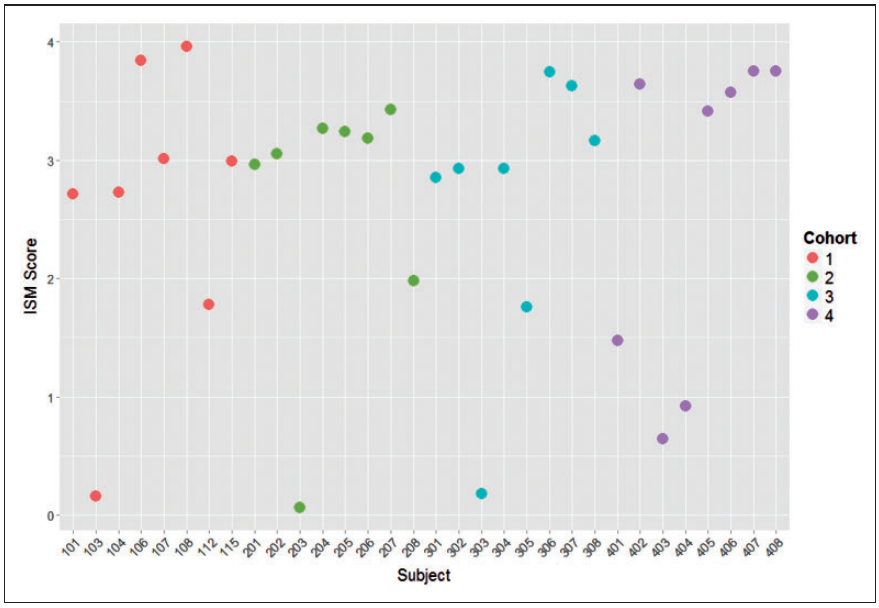

Figure 3 The baseline interferon signature metric score for each subject in the study, grouped by cohort, is shown above.

treatment of bronchitis. A heat map showing the baseline gene expression profile of the 32 study subjects as compared to 20 health volunteers is shown in Supplementary Figure 1.

Autoantibody profiles

During the study, modest decreases in RNA autoantibodies were observed in five drug-treated subjects (112, 201, 305, 307 and 402) and one subject in the placebo group (405). Decreases of 10–20% were observed for Sm, RNP, and Ro52 in drug-treated subjects 112, 201, 305; a decrease in Sm of 60% was observed in subject 402. This subgroup of drug-treated subjects also experienced improvements in disease symptoms, as measured by decreases in SLEDAI score. Subject 307, also in the drug-treated group, had an approximately 20% decrease in Sm, smRNP, Ro, and U1RNP autoantibodies, and this was accompanied by a

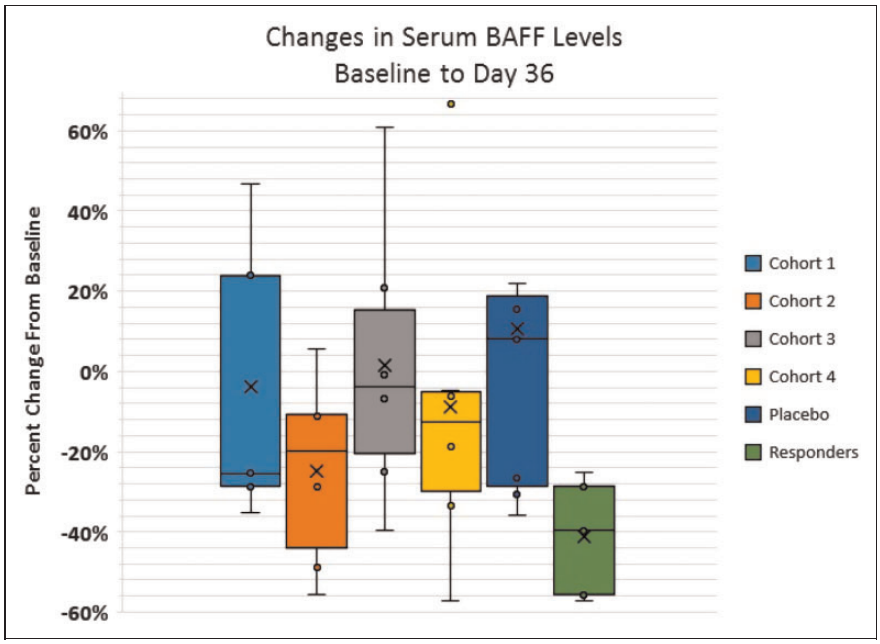

Figure 4 Serum B-cell activating factor levels were quantitated at baseline and Day 36, one week following the last dose of RSLV-132. Data are grouped by cohort and drug treatment group. The responder subgroup is a group of five RSLV-132 treated subjects who had clinical improvements and decreases in autoantibodies.

Table 3 Summary of SLE Disease Activity Index (SLEDAI) measurements

	Cohort 1	*Cohort 2*	*Cohort 3*	*Cohort 4*	*Placebo*
Dosing regimen	0.6 mg/kg × 1 0.3 mg/kg × 4	2.0 mg/kg × 1 1.0 mg/kg × 2	6.0 mg/kg × 1 3.0 mg.kg × 2	10.0 mg/kg × 3	
Baseline SLEDAI	5.2 ± 4.4	3.0 ± 3.0	2.7 ± 1.6	2.3 ± 2.9	3.8 ± 2.5
Day 43 SLEDAI	2.4 ± 4.3	1.7 ± 2.0	3.0 ± 1.1	1.7 ± 2.7	2.8 ± 2.8
SLEDAI improvement (evaluable subjects)	4/4 (100%)	1/4 (25%)	1/5 (20%)	1/3 (33%)	2/6 (33%)

significant improvement in rash (although not completely resolved so there was no change in SLEDAI). Subject 405 in the placebo group experienced an approximately 40% decrease in SmRNP and U1RNP which was accompanied by an increase in disease activity and SLEDAI score.

Disease activity

The average SLEDAI score at baseline for the 32 study subjects was 3.5 ± 2.9. Of the 32 study subjects, nine (28%) had a SLEDAI score of zero at baseline; the mean baseline SLEDAI for the remaining 23 subjects was 4.7 ± 2.3. The distribution of SLEDAI scores by cohort is shown in Table 3. Of the 31 subjects who completed the study 70% (22/31) had a SLEDAI > 0 and were therefore evaluable. Of the evaluable subjects, 44% (7/16) in the RSLV-132 treated group experienced an improvement in SLE disease symptoms and a decrease in SLEDAI score, while 33% (2/6) subjects in the placebo group had an improvement in SLEDAI score. There were a total of three subjects in the study who experienced a worsening of SLE symptoms and an increase in SLEDAI score between baseline and Day 43; 9% (2/23) were in the RSLV-132 treated group and 13% (1/8) were in the placebo group.

Interferon-inducible protein analysis

To evaluate the impact of RSLV-132 on serum interferon (IFN)-inducible protein levels, BAFF was measured at baseline and one week after the last dose of RSLV-132 on Day 36. Overall, among the RSLV-132 treated group there was an average 9% decrease in BAFF levels from baseline to Day 36, while the placebo group had an average increase in BAFF of 11%. The mean BAFF level changes between baseline and Day 36 for each

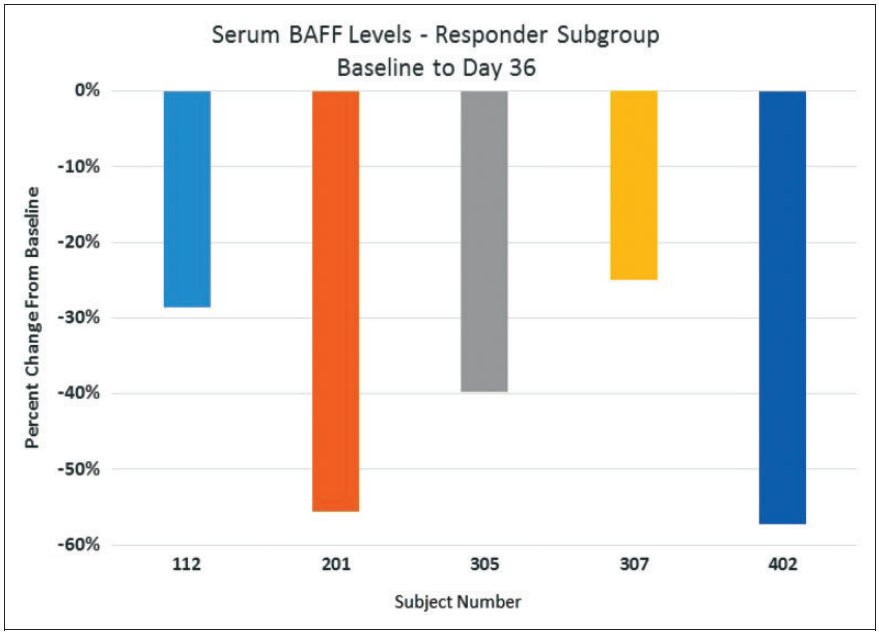

Figure 5 Serum B-cell activating factor levels were quantitated at baseline and Day 36, one week following the last dose of RSLV-132. The responder subgroup is a group of five RSLV-132 treated subjects who had clinical improvements and decreases in autoantibodies.

cohort were; −4% (cohort 1), −25% (cohort 2), +1% (cohort 3), and −9% (cohort 4). A subgroup of five RSLV-132 treated subjects who experienced decreases in autoantibodies and decreases in SLEDAI scores demonstrated a more significant decrease in BAFF levels with an average decrease of 41% (Figure 4). Decreases in BAFF levels among this responder subgroup ranged from 29% to 57% (Figure 5).

Discussion

A large body of evidence points to the importance of RNA in driving inflammation through a cascading activation of dendritic cells, B-cells, TLRs, IFN production, and subsequent activation of multiple immune cell types.[15–20] In healthy individuals there is appreciable circulating endogenous RNase activity; while the role of circulating RNase has not been well defined, it may help prevent inflammation due to circulating RNA resulting from apoptotic cell debris.[21] However, in SLE patients there are two events which overcome this protective mechanism: the inability to effectively clear the apoptotic cellular debris swamping the endogenous RNase, and the presence of autoantibodies which when bound to antigens containing RNA serve as delivery vehicles, bringing the RNA into contact with intracellular TLRs via Fc-mediated uptake into immune cells.[22] RNA is therefore positioned at the furthest upstream step in the inflammatory cascade, and preventing this initial stimulus would be expected to prevent activation and amplification of multiple downstream cell types and inflammatory cytokines. RSLV-132 was designed specifically to interrupt the inflammatory cascade by removing the RNA from circulation before it can be delivered via autoantibodies to immune cell activation systems. The present study describes the first clinical trial of a novel nuclease therapy, RSLV-132, in subjects with SLE. The study was designed to examine the safety and tolerability of RSLV-132 in patients with SLE and enrolled 32 subjects with mild, stable disease. The drug was administered via intravenous infusion with five (cohort 1) or three (cohorts 2–4) doses given over one month. The compound demonstrated an excellent safety profile with primarily grade 1 AEs, the majority of which were not related to the study drug. There was one SAE in a subject with a history of cholecystitis who required a cholecystectomy. There were no deaths in the study. All but one of the subjects completed the one month of dosing. There were no infusion reactions noted in any of the subjects in the study.

A validated anti-drug antibody assay was used to test for antibodies against RSLV-132 and subjects were tested at baseline, once during the study and

at the end of study visit. None of the 32 subjects in the study tested positive for anti-drug antibodies at any point in the study. While data from larger studies of longer duration are needed, this is an encouraging result. The serum exposure of RSLV-132 was dose proportional and the competitive ELISA and RNase methods were in very good agreement making it possible to monitor RNase activity during the study. If anti-RSLV-132 antibodies were to be encountered, measuring the RNase activity would provide information about potential neutralizing effects. Since the RNase catalytic activity of RSLV-132 is the central mechanism of action, having a means to monitor this moiety will be very informative as dose ranging studies are undertaken. The half-life of RSLV-132 was approximately 19 days which may support once monthly dosing. The RNase catalytic activity was linearly increased through the dosing range, and the serum levels of RNase were increased from 2–128 μg/mL at C_{max}, to 0.3–5 μg/mL at C_{min}. Thus, there appears to be no endogenous inhibitor of RNase activity. The PK data are fit well using a two-compartment model, which may indicate the drug is distributing to various extra-circulatory spaces such as skin, vasculature or joints. It is possible this is a function of the glycan structure of the molecule; additional studies are required to determine the exact distribution of the molecule. Given the novel mechanism of action of RSLV-132, several different pharmacodynamic parameters were explored, including analysis of autoantibody levels, gene expression, and serum protein analysis. Modest decreases in RNA-containing autoantibodies were noted in a small subgroup of subjects that had clinical improvements. The gene expression analysis involved examining 180 transcripts that were thought to be involved in various inflammatory pathways. The gene array was organized into modules containing clusters of genes that were thought to be involved in certain pathways, such as interferon-regulated genes, B-cell genes, and neutrophil genes.[23] While there were changes in gene expression in the B-cell and neutrophil modules among some drug-treated subjects, there was no cohesive pattern observed. This likely reflects the small study size, large variation in disease activity among the subjects, the large range in dose, and the short treatment period. The array will be deployed in a larger clinical study with a more homogenous patient population. For the interferon gene module, ISM scores were calculated for all subjects at baseline and Day 43 of the study. Although the majority of subjects (74%) had mild SLE with a baseline SLEDAI ≤ 4, and a significant fraction of subjects (29%) were symptom free with a baseline SLEDAI of zero, the majority of subjects were IFN-signature positive with an average ISM score of three. With the exception of two drug-treated subjects who had a decrease in ISM score, the ISM scores were quite stable throughout the study. The mechanism of action of RSLV-132 suggested an attenuation of the interferon signature may have been observed if the biomarker were driven primarily by interferon-α. However, it appears that this gene signature may be driven by multiple type I interferons as well as type II interferon.[24] Indeed, clinical studies in SLE patients with an anti-interferon-α monoclonal antibody that directly neutralizes the cytokine, have shown only weak decreases in the interferon signature (6–25%), despite demonstrating improvements in disease activity.[24,25] Future studies on larger numbers of patients with more active disease will determine the ultimate utility of the IFN gene signature as a pharmacodynamic marker for RSLV-132. Serum interferon-inducible proteins were also measured at baseline and Day 36, focusing on BAFF. Decreases in serum BAFF were observed in the drug-treated cohorts (overall −9%) versus the placebo group (+11%). However, a subgroup of subjects that tended to have more active disease, and experienced clinical improvements, demonstrated significant decreases in BAFF levels at Day 36 (29–57%). In conclusion, RSLV-132 was safe and well tolerated in this cohort of lupus subjects. A phase 2 study is currently ongoing to further explore the potential therapeutic utility of this novel therapy.

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Declaration of Conflicting Interests

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References

1 Shao WH, Cohen PL. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. *Arthritis Res Ther* 2011; 13: 202.
2 Arbuckle MR, McClain MT, Rubertone MV, *et al*. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003; 349: 1526–1533.
3 Von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum* 1995; 24: 323–358.
4 Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. *Immunol Rev* 2010; 233: 126–145.
5 Lau CM, Broughton C, Tabor AS, *et al*. RNA-associated autoantigens activate B cells by combined B cell receptor/Toll-like receptor 7 engagement. *J Exp Med* 2005; 202: 1171–1177.
6 Vollmer J, Tluk S, Schmitz C, *et al*. Immune stimulation mediated by autoantigen binding sites within small nuclear RNAs involves Toll-like receptors 7 and 8. *J Exp Med* 2006; 202: 1575–1585.
7 Leadbetter EA, Rifkin IR, Hohlbaum AM, *et al*. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002; 416: 603–607.
8 Lovgren T, Eloranta ML, Bave U, Alm GV, Ronnblom L. Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum* 2004; 50: 1861–1872.
9 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271–1277.
10 Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. *Arthritis Rheum* 1997; 40: 1725.
11 Kennedy WP, Maciuca R, Wolslegel K. Association of the interferon signature metric with serological disease manifestations but not global activity scores in multiple cohorts of patients with SLE. *Lupus Sci Med* 2015; 2: e000080.
12 Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 2004; 93: 2645–2648.
13 Sahin S, Benet LZ. The operational multiple dosing half-life: A key to defining drug accumulation in patients and to designing extended release dosage forms. *Pharm Res* 2008; 25: 2869–2877. DOI: 10.1007/s11095-008-9787-9.
14 Hua J, Kirou K, Lee C, Crow M. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. *Arthritis Rheum* 2006; 54: 1906–1916.
15 Ahlin E, Mathsson L, Eloranta ML, *et al*. Autoantibodies associated with RNA are more enriched than anti-dsDNA antibodies in circulating immune complexes in SLE. *Lupus* 2012; 21: 586–595.
16 Barrat FJ, Elkon KB, Fitzgerald KA. Importance of nucleic acid recognition in inflammation and autoimmunity. *Annu Rev Med* 2016; 67: 323–336. DOI: 10.1146/annrev-med-052814-023338.
17 Bave U, Vallin H, Alm G, Ronnblom L. Activation of natural interferon-alpha producing cells by apoptotic U937 cells combined with lupus IgG and its regulation by cytokines. *J Autoimmun* 2001; 17: 71–80.
18 Marshak-Rothstein A, Rifkin IR. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu Rev Immunol* 2007; 25: 419–441.
19 Santer DM, Yoshio T, Minota S, Moller T, Elkon KB. Potent induction of IFN-alpha and chemokines by autoantibodies in the cerebrospinal fluid of patients with neuropsychiatric lupus. *J Immunol* 2009; 182: 1192–1201.
20 Weickmann JL, Glitz DG. Human ribonucleases. Quantitation of pancreatic-like enzymes in serum, urine, and organ preparations. *J Biol Chem* 1982; 257: 8705–8710.
21 Poon IK, Lucas CD, Rossi AG. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol* 2014; 14: 166–180.
22 Chaussabel D, Quinn C, Shen J, *et al*. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. *Immunity* 2008; 29: 150–164.
23 Chiche L, Jourde-Chiche N, Whalen E, *et al*. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol* 2014; 66: 1583–1595.
24 Khamashta M, Merrill JT, Werth VP, *et al*. Sifalimumab, an anti-interferon-alpha monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomized, double-blind, placebo-controlled study. *Ann Rheum Dis* 2016; 75: 1909–1916. DOI: 10.1136/annrheumdis-2015-208562.
25 Morehouse C, Brohawn P, Higgs B, *et al*. Pharmacokinetics of sifalimumab and target modulation of a type I interferon gene signature in patients with moderate to severe systemic lupus erythematosus. *Ann Rheum Dis* 2015; 74: 946. DOI: 10.1136/annrheumdis-2015-eular.4529.